SEQUENCE LISTING

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<120> Novel Human Cancer Antigen NY ESO-1/CAG-3 and Gene Encoding Same

<130> 20264269PC

<140> PCT/US98/19609
<141> 1998-09-21

<150> US60/061,428
<151> 1997-10-08

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<170> PatentIn Ver. 2.0

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Reverse Transcriptase-PCR

Total RNA was extracted from tumor cell lines as described above. Five hundred nanograms of total RNA was used for conversion of RNA to cDNA by avian myeloblastosis virus (AMV) reverse transcriptase. cDNA was then amplified by PCR using the ESO-P2 (5'GCGGCTTCAGGGCTGAATGGATG) (SEQ. ID No. 105) and ESO-P5 (5'-AAGCCGTCCTCCTCCAGCGACA) (SEQ. ID No. 106) primers and One-Step RT-PCT system (Life Technologies). PCR products were amplified under denaturation conditions at 94°C for 30 s, annealing at 55° for 30 s, extension at 72° for 3 min for 40 cycles, and final elongation at 72° for 10 min. PCR products were analyzed on a 3% agarose gel.

Cytotoxic lysis assays

Cytolytic assay was done as previously described (Kawakami, Y et al., 1994, *Proc. Natl. Acad. Sci.* USA 91:6458-62). Briefly, the target cells were labeled with chromium for 90 min. After washing three times, the cells were incubated with peptides at a concentration of 1 μg/ml for 90 min. The cells were washed again, counted, and then mixed with TIL586 or CTL clone 5 at the indicated ratio of effector : targets (E : T). Chromium release was measured after 4 h incubation. The peptides were synthesized by a solid-phase method using a peptide synthesizer (Model AMS 422, Gilson Co., Inc., Worthington, OH). Some peptides were purified by HPLC and had greater than 98% in purity. The mass of some peptides was confirmed by mass spectrometry analysis. For titration of the CAG-3 peptide recognized by TIL586, 586EBV B cells were incubated with various concentrations of the purified CAG-3 peptide or portion thereof. Percentage of specific lysis was determined from the equation (A-B)/(C-B) X 100 where A is lysis of 586EBV B cells by TIL586 or clone 5 in the presence of a peptide, B is spontaneous release from 586EBV B cells in presence of the same peptide but in the absence of effector cells, and C is the maximum chromium release. Cold target inhibition of cytolysis was performed using ^{51}Cr-labeled 586mel or 624mel cells as "hot" targets and 586EBV B and T2 cells pulsed with peptides as "cold" targets.